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(54) VITAL TISSUE FOR TENDON OR LIGAMENT AND PROCESS FOR PRODUCING THE SAME

(57) A biocompatible tissue for tendons or ligaments which enables rapid postoperative fixation to bone and early recovery owing to its enhanced biocompatibility and a method for producing such tissue. The biocompatible tissue for tendons or ligaments includes a tendon or ligament tissue substrate and a calcium phosphate compound. The calcium phosphate compound is fixed at least to a surface of the tendon or ligament tissue substrate. The method includes the step of alternately immersing the substrate in a calcium solution and in a phosphate solution to produce and fix the calcium phosphate compound.

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Description

[0001] The present invention relates to a biocompatible tissue for tendons or ligaments which can be used in reconstructive surgery of tendons and ligaments and enables rapid postoperative fixation to bone and early recovery owing to its enhanced biocompatibility. The present invention also relates to a method for producing such a biocompatible tissue.

[0002] Reconstructive materials are generally used in reconstructive surgery of tendons and ligaments in order to minimize damage to healthy self-tissue and minimize the amount of self-tissue removal. Known techniques of reconstructive surgery include a technique in which nondegradable, highly strong synthetic high molecular material as a reconstructive material is used as substitutes for self-tissue, a technique in which artificial ligaments composed of self-tissue and synthetic high molecular material (*e.g.*, polypropylene) are used to bear some of the mechanical stress that applies to the reconstructed ligaments until self-tissue is regenerated so as to enable early post-operative rehabilitation, and a technique in which biodegradable artificial ligaments are used to serve as ligaments while self-tissue is being induced.

[0003] However, all of these techniques are accompanied by a problem of the insufficient fixation rate or strength of the tendons or ligaments to bone after reconstructive surgery.

[0004] For this reason, reconstructive surgery which relies on self-transplantation rather than on use of artificial ligaments has become increasingly common. For example, a patellar tendon with a fragment of bone attached thereto or a hamstring tendon is used as self-tissue in reconstructive surgery of anterior cruciate ligament.

[0005] The purpose of using the patellar tendon with its associated bone fragment is to permit the use of interference screws by taking advantage of the innate attachments of bone/ligaments, thereby facilitating fast reconstruction. This type of reconstructive surgery provides better initial fixation and enables coaptation within a bone tunnel at an early stage. However, such reconstructive surgery is associated with problems such as relatively frequent occurrence of complications such as post-operative knee stretching impairment, knee pain and fracture of patella and necessity of skill for screw fixation.

[0006] The advantage of using hamstring tendons is that there are few complications associated with the removal of the self-tendons and that the procedure is relatively simple and has a large degree of freedom. However, this approach also has drawbacks such as weak initial fixation strength resulting in a prolonged fixation time of bone to soft tissue, and a extended rehabilitation period.

[0007] For obtaining better fixation of bone with soft tissue, i.e. tendons or ligaments, a method is proposed in which collagen and BMP (bone morphogenic protein) are used to induce generation of bone formation at an early stage in the region where the bone abuts the soft tissue. However, the method has not result in success.

[0008] It is a well-known fact that calcium phosphate compounds have excellent biocompatibility, bone inducibility, and bone conductibility and may be used as substitutes for bone and as prosthetic materials for medical use. However, techniques have yet to be established for artificially fixing fine powders of calcium phosphate compounds such as hydroxyapatite and tricalcium phosphate in soft tissue such as tendons and ligaments.

[0009] Accordingly, it is an objective of the present invention to provide a biocompatible tissue for tendons or ligaments which enables rapid postoperative fixation to bone and early recovery owing to its enhanced biocompatibility, and a method for producing such biocompatible tissue.

[0010] It is another objective of the present invention to provide a method for producing a biocompatible tissue for tendons or ligaments which enables rapid postoperative fixation to bone and early recovery owing to its enhanced biocompatibility in a simple manner and in a short period of time.

[0011] The present inventors have made extensive research and found out that, by alternately immersing a substrate for tendon or ligament tissue in a calcium solution and in a phosphate solution, the highly biocompatible calcium phosphate compound such as hydroxyapatite can be produced and firmly fixed on the surface of or within the substrate in a short period of time. The present inventors have further found out that this production method through the alternate immersion process makes it easy to control the amount of the calcium phosphate compound produced and to control the fixation of the calcium phosphate compound while maintaining physical properties of the substrate required for the tissue for tendons or ligaments, thereby completing the present invention.

[0012] According to the present invention, there is provided a biocompatible tissue for a tendon or a ligament comprising a tendon or ligament tissue substrate and a calcium phosphate compound, wherein the calcium phosphate compound is fixed at least to a surface of the tendon or ligament tissue substrate.

[0013] According to the present invention, there is also provided a method for producing the above-described biocompatible tissue for a tendon or a ligament. The method includes the step of alternately immersing the tendon or ligament tissue substrate in a calcium solution containing calcium ions but substantially no phosphate ions (which may be referred to hereinafter as a calcium solution (A)) and in a phosphate solution containing phosphate ions but substantially no calcium ions (which may be referred to hereinafter as a phosphate solution (B)), to produce and fix the calcium phosphate compound at least on the surface of the substrate.

[0014] According to the present invention, there is further provided a method for treating a tendon or a ligament including the steps of collecting from an animal including a human a self-tissue for tendons or ligaments as a substrate, alternately immersing the substrate in the calcium solution (A) and in the phosphate solution (B) to produce and fix the calcium phosphate compound at least on a surface of the substrate to obtain the above-described biocompatible tissue for the tendon or ligament and implanting the resulting biocompatible tissue for the tendon or ligament in a defective tendon or ligament of an animal including a human.

[0015] The present invention will now be described in detail.

[0016] The biocompatible tissue of the present invention for a tendon or a ligament, which can be used as substitutes for tendons and ligaments in reconstructive surgery of tendons and ligaments in animals including humans, includes a tendon or ligament tissue substrate (which may be referred to hereinafter as substrate) and a calcium phosphate compound.

[0017] The substrate may be any substrate that can serve as a tendon or a ligament of an animal including a human. For example, the substrate composed of one or more of materials selected from the group consisting of an artificial tendon, an artificial ligament, a natural tendon of an animal, and a natural ligament of an animal may be used. The substrate using the natural tendon of an animal or the natural ligament of an animal are particularly preferred. Any known artificial tendons or ligaments may serve as the artificial tendons or ligaments of the present invention while natural tendons and ligaments surgically extracted from animals may be used as the natural tendons and ligaments of animals of the present invention. Among natural tendons and ligaments, self-tissue is particularly preferred. These materials may be subjected to various medically acceptable treatments, or they may include other materials provided that the other materials do not adversely affect the intended purpose of the invention.

[0018] In the tendon or ligament tissue of the present invention, the calcium phosphate compound to be fixed to the substrate may be any calcium phosphate compound obtained through a reaction between a phosphate and calcium. Examples thereof may include hydroxyapatite, tricalcium phosphate, octacalcium phosphate and mixtures thereof. Of these, hydroxyapatite is the most preferred since it has the highest compatibility with living bodies. While the calcium phosphate compounds in the present invention do not include natural bone, the tendon or ligament tissue of the present invention may include natural bone.

[0019] In the biocompatible tissue for the tendon or ligament of the present invention, the calcium phosphate compound is fixed at least to a surface of the substrate. It is preferred that the calcium phosphate compound be fixed also within the substrate in order to facilitate fast fixation of the tissue to bone after reconstructive surgery. The term "fixed" as used herein means that the calcium phosphate compound does not come off the substrate through the regular process of washing the substrate several times. In one preferred embodiment of the present invention which is provided by a method of the present invention described below, the calcium phosphate compound is directly fixed to the substrate without using adhesives, which may affect biocompatibility.

[0020] In the biocompatible tissue for the tendon or ligament of the present invention, the calcium phosphate compound may be fixed in an amount of 0.5 to 20wt%, in particular 1 to 10wt%, with respect to the total amount of the biocompatible tissue for the tendon or ligament in order to ensure fast fixation of the tissue to bone although the amount may not necessarily fall in the specified ranges. Further, the amount of hydroxyapatite in the calcium phosphate compound may preferably be from 50 to 100wt% so that hydroxyapatite constitutes the primary component of the calcium phosphate compound.

[0021] When necessary, the biocompatible tissue for the tendon or ligament of the present invention may be subjected to various medically acceptable treatments or they may include other materials provided that the other materials do not adversely affect the intended purpose of the invention.

[0022] The biocompatible tissue for the tendon or ligament of the present invention may be used in reconstructive surgery of tendons or ligaments. In cases where self-tissue is used as the substrate, the self-tissue to serve as tendons or ligaments may be surgically collected, and the biocompatible tissue for the tendon or ligament of the present invention may be prepared by fixing the calcium phosphate compound to the collected self-tissue during reconstructive surgery. The biocompatible tissue so prepared may then be used in the reconstructive surgery.

[0023] Thus, the present invention also provides a method for treating tendons or ligaments. The method involves collecting from an animal including a human self-tissue for a tendon or ligament to serve as the substrate, producing the calcium phosphate compound and fixing it at least to a surface of the substrate through the alternate immersion process, which is described in detail later, to obtain the biocompatible tissue for the tendon or ligament, and implanting the biocompatible tissue for the tendon or ligament in a defective tendon or ligament of an animal including a human.

[0024] In the method for producing the biocompatible tissue for the tendon or ligament of the present invention, it is preferred that the calcium phosphate compound be readily fixed to the substrate directly and in a short period of time. For example, the method may include the step of alternately immersing a substrate for the tendon or ligament tissue in a calcium solution (A) and in a phosphate solution (B) (which may be referred to hereinafter as the alternate immersion step) to produce the calcium phosphate compound and fix it at least to the surface of the substrate, but is not limited thereto.

[0025] The calcium solution (A) used in the aforementioned alternate immersion step may be an aqueous solution containing calcium ions but substantially no phosphate ions. In the presence of phosphate ions, the rate at which hydroxyapatite is produced may be decreased. For this reason, the calcium solution (A) may be formed as an aqueous solution containing calcium ions but no phosphate ions. Examples of the calcium solution may include an aqueous solution of calcium chloride, an aqueous solution of calcium acetate and buffered solutions thereof. Examples of the buffering agent may include a tris(hydroxymethyl)aminomethane-HCl buffer (referred to hereinafter as Tris buffer), but is not limited thereto.

[0026] The calcium solution (A) preferably has a calcium ion concentration of 0.1 to 40wt%, particularly 1 to 10wt%, in view of the production rate of the calcium phosphate compound and the production efficiency of the compound. The concentration of calcium ions less than 0.1wt% is not preferable since the amount of calcium phosphate compound produced in each alternate immersion step may be so small that the step may need to be repeated many times to produce calcium phosphate compound in desired amounts.

[0027] The pH of the calcium solution (A) is preferably adjusted to a value of 7 to 9 using for example a Tris buffer in order to reduce the risk of damaging living tissue though the pH is not necessarily limited to the specified range.

[0028] The phosphate solution (B) used in the alternate immersion step may be an aqueous solution containing phosphate ions but substantially no calcium ions. In the presence of calcium ions, the rate at which hydroxyapatite is produced may be decreased. For this reason, the phosphate solution (B) may be formed as an aqueous solution containing phosphate ions but no calcium ions. Examples of the phosphate solution may include an aqueous solution of sodium dihydrogenphosphate, an aqueous solution of disodium hydrogenphosphate, an aqueous solution of diammonium hydrogenphosphate, and buffered solutions thereof. Use of the aqueous solution of dihydrogensodium phosphate or a buffered solution thereof is particularly preferred in order to reduce damage to living tissue and enhance the production of hydroxyapatite. Examples of the buffering agent may include a Tris buffer, but is not limited thereto.

[0029] The phosphate solution (B) preferably has a phosphate ion concentration of 0.1 to 20wt%, particularly 0.5 to 10wt%, in view of the production rate of the calcium phosphate compound and the production efficiency of the compound. The concentration of phosphate ions less than 0.1wt% is not preferable since the amount of calcium phosphate compound produced in each alternate immersion step may be so small that the step may need to be repeated many times to produce calcium phosphate compound in desired amounts.

[0030] The pH of the phosphate solution (B) is preferably adjusted to a value of 7 to 9 using for example a Tris buffer in order to reduce the risk of damaging living tissue though the pH is not necessarily limited to the specified range.

[0031] The calcium solution (A) and the phosphate solution (B) may contain other types of ions provided that the other ions do not adversely affect the intended purpose of the invention.

[0032] The methods for immersing the substrate in the calcium solution (A) and the phosphate solution (B) in the alternate immersion step may include:

(1) performing one or more cycles of a series of operation of immersing the substrate first in the calcium solution (A) and then in the phosphate solution (B), and

(2) performing one or more cycles of a series of operation of immersing the substrate first in the phosphate solution (B) and then in the calcium solution (A).

[0033] The amount of the calcium phosphate compound produced can be increased by increasing the number of repeats of the operation. The number of repeats of the operation may generally be from 1 to 20 times. When the series of the operation (1) is repeated more than once, the final immersion is not necessarily the immersion in the phosphate solution (B) and may be in the calcium solution (A). Similarly, when the series of the operation (2) is repeated more than once, the final immersion is not necessarily the immersion in the calcium solution (A) and may be in the phosphate solution (B).

[0034] The time for which the substrate is immersed in the calcium solution (A) and the phosphate solution (B) may be properly adjusted in view of the rate and efficiency of the production of the calcium phosphate compound and the ion concentration of each solution. In general, the total immersion time is preferably selected to be several tens of seconds to one hour, in particular, 1 to 30 minutes. When the series of the operation of immersing the substrate in the calcium solution (A) and the phosphate solution (B) is repeated, the immersion time per cycle of the immersion step may be suitably selected in consideration of the preferred total immersion time.

[0035] The temperature of each solution during the immersion of the substrate in the solution is properly selected in view of the rate and the efficiency of the production of the calcium phosphate compound and may generally be selected to be a temperature of 15 to 40°C.

[0036] The alternate immersion step preferably includes in each of the above-described cycle a step of removing the calcium solution (A) remaining on the substrate, after the substrate is immersed in the calcium solution (A) and before the substrate is immersed in the phosphate solution (B), and/or, a step of removing the phosphate solution (B) remaining on the substrate, after the substrate is immersed in the phosphate solution (B) and before the substrate is

immersed in the calcium solution (A), in order to ensure firm fixation of the calcium phosphate compound and removal of excess calcium ions and phosphate ions.

[0037] Any process that can remove calcium ions or phosphate ions remaining on the surface of the substrate may be employed as the process of removing the calcium solution (A) or the phosphate solution (B) remaining on the substrate. For example, rinsing with a rinsing liquid such as sterilized water or saline is preferred. While the rinsing process may only involve simply immersing the substrate in the rinsing liquid, it may also involve gently stirring the rinsing liquid or gently vibrating the substrate. Preferably, the temperature of the rinsing liquid may be selected to be in the range of 15 to 40°C.

[0038] The production method of the present invention may include other steps so long as the method includes the above-described alternate immersion step and the biocompatible tissue for the tendon or ligament of the present invention is achieved.

[0039] While the present invention is hereinbelow described in further detail with reference to Examples, the present invention is not limited thereto. As used herein, the sign % always signifies wt% unless otherwise specified.

Example 1

[0040] Free tendons were surgically removed from a hind leg of a sacrificed Japanese White rabbit. At room temperature, the removed free tendons were immersed for 5 minutes in an aqueous solution of calcium chloride (24°C) having a calcium ion (Ca^{2+}) concentration of 0.8% and preconditioned to pH7.4 using a Tris buffer. Subsequently, the tendons were immersed in saline for 30 seconds and then in an aqueous solution of disodium hydrogenphosphate having a phosphate ion (PO_4^{2-}) concentration of 1.1% for 5 minutes. This process was performed once or repeated 3, 6 and 8 times, and the tendons were rinsed with saline for 5 minutes to prepare biocompatible tissues for use as tendons.

[0041] A visual observation of each biocompatible tissue for the tendon revealed that the tissue turned increasingly white as the number of repeats of the alternate immersion was increased. Also, an observation of cross-sections taken along a line perpendicular to longitudinal axis of the biocompatible tissue for the tendon revealed that the color change to white also occurred within the tissue for the tissue subjected to 3 times or more of the alternate immersion.

[0042] The resulting biocompatible tissue for the tendon was freeze-dried and the amounts of samples produced, *i.e.*, the amounts of the calcium phosphate compound produced, were measured using a thermo-analyzer (DTA-TG) (manufactured by RIGAKU Corporation, model No. TG8120). The results are shown in Table 1.

[0043] Further, the samples obtained by repeating the alternate immersion 8 times were baked in an electric furnace at 1200°C and were then powdered. It was determined that the resulting powder contained hydroxyapatite as its principal component by measuring the powder with a powder X-ray diffraction analyzer (XRD) (manufactured by Phillips Petroleum Company, model No. PW1729). It is thus confirmed that hydroxyapatite was the primary component of the white products.

Example 2

[0044] Free tendons were surgically removed from a hind leg of a sacrificed Japanese White rabbit. At room temperature, the removed free tendons were immersed in an aqueous solution of calcium chloride (24°C) having a calcium ion (Ca^{2+}) concentration of 4.8% for 2 minutes. Subsequently, the tendons were immersed in a saline for 30 seconds and then in an aqueous solution of disodium hydrogenphosphate having a phosphate ion (PO_4^{2-}) concentration of 6.8% for 2 minutes. This process was performed once or repeated twice, 6 and 8 times, and the tendons were rinsed with saline for 5 minutes to prepare biocompatible tissues for use as tendons.

[0045] A visual observation of each biocompatible tissue for the tendon revealed that the tissue turned increasingly white as the number of repeats of the alternate immersion was increased. Also, an observation of cross-sections taken along a line perpendicular to longitudinal axis of the biocompatible tissue for the tendon revealed that the color change to white also occurred within the tissue for the tissue subjected to 6 times or more of the alternate immersion.

[0046] The resulting biocompatible tissue for the tendon was freeze-dried and the amount of the calcium phosphate compound was determined as in Example 1. The results are shown in Table 1.

Table 1

	Amount of calcium phosphate produced at each immersion number (%)				
	Immersion once	Immersion twice	Immersion 3 times	Immersion 6 times	Immersion 8 times
Example 1	0.9	-	3.7	6.8	8.5
Example 2	1.1	4.2	-	7.4	9.0

Example 3

[0047] Free tendons were surgically removed from a hind leg of a sacrificed Japanese White rabbit. At room temperature, the removed free tendons were immersed for 5 minutes in an aqueous solution of calcium acetate (26°C) having a calcium ion (Ca^{2+}) concentration of 1.6% preconditioned to pH7.4 using a Tris buffer. Subsequently, the tendons were immersed in saline for 30 seconds and then in an aqueous solution of disodium hydrogenphosphate having a phosphate ion (PO_4^{2-}) concentration of 2.3% for 5 minutes. This process was repeated 5 times and the tendons were rinsed with saline for 5 minutes to prepare a biocompatible tissue for use as a tendon. A visual observation of the biocompatible tissue for tendons revealed that the tissue turned white on its surface as well as its interior.

[0048] The resulting biocompatible tissue for the tendon was freeze-dried, and the amount of the calcium phosphate compound was measured as in Example 1 and was determined to be 7.5%.

[0049] The biocompatible tissue for the tendon or ligament of the present invention is useful in reconstructive surgery of tendons or ligaments since the calcium phosphate compound, fixed at least to a surface of the tendon or ligament tissue substrate, does not come off the substrate easily. Further, the biocompatible tissue for tendon or ligament of the present invention enables fast induction of bone tissue formation as well as firm fixation of the tissue to bone after surgery based on the biocompatibility of the fixed calcium phosphate compound.

[0050] The production method of the present invention, which involves the alternate immersion process, makes it possible for the calcium phosphate compound to be produced and fixed directly to the substrate. Furthermore, since the method of the present invention may be performed with the simple steps of the alternate immersion, the method makes it easy to control the fixation of the calcium phosphate compound while maintaining physical properties of the substrate required for the tissue for tendons or ligaments. It also enables the production and fixation of the calcium phosphate compound in a short period of time. Thus, the ligament or tendon tissue of the present invention can be prepared while reconstructive surgery is being performed.

Claims

1. A biocompatible tissue for a tendon or a ligament comprising a tendon or ligament tissue substrate and a calcium phosphate compound, the calcium phosphate compound being fixed at least to a surface of the tendon or ligament tissue substrate.

2. The biocompatible tissue for the tendon or ligament according to claim 1, wherein the tendon or ligament tissue substrate is a substrate composed of one or more of materials selected from the group consisting of an artificial tendon, an artificial ligament, a natural tendon of an animal, and a natural ligament of an animal.

3. The biocompatible tissue for the tendon or ligament according to claim 1, wherein the calcium phosphate compound comprises hydroxyapatite.

4. A method for producing the biocompatible tissue for the tendon and ligament according to claim 1, the method comprising the step of alternately immersing the tendon or ligament tissue substrate in a calcium solution containing calcium ions but substantially no phosphate ions and in a phosphate solution containing phosphate ions but substantially no calcium ions, to produce and fix the calcium phosphate compound at least on the surface of the substrate.

5. The method according to claim 4, wherein the tendon or ligament tissue substrate is a substrate composed of one or more of materials selected from the group consisting of an artificial tendon, an artificial ligament, a natural tendon of an animal, and a natural ligament of an animal.

6. The method according to claim 4, wherein the calcium solution containing calcium ions but substantially no phosphate ions has a calcium ion concentration of 0.1 to 40wt% and the phosphate solution containing phosphate ions but substantially no calcium ions has a phosphate ion concentration of 0.1 to 20wt%.

7. The method according to claim 4, wherein the step of alternately immersing the tendon or ligament tissue substrate in the calcium solution and in the phosphate solution further includes the step of removing the calcium solution remaining on the tendon or ligament tissue substrate after the substrate is immersed in the calcium solution and before the substrate is immersed in the phosphate solution and the step of removing the phosphate solution remaining on the tendon or ligament tissue substrate after the substrate is immersed in the phosphate solution and before the substrate is immersed in the calcium solution.

8. The method according to claim 4, wherein a condition in which the tendon or ligament tissue substrate is alternately immersed in the calcium solution and in the phosphate solution is adjusted so that the calcium phosphate compound produced and fixed includes hydroxyapatite.

9. A method for treating a tendon or a ligament comprising the steps of collecting from an animal including a human self-tissue for a tendon or a ligament to serve as a substrate, alternately immersing the substrate in a calcium solution containing calcium ions but substantially no phosphate ions and in a phosphate solution containing phosphate ions but substantially no calcium ions to produce and fix a calcium phosphate compound at least on a surface of the substrate to obtain the biocompatible tissue for tendon or ligament of claim 1, and implanting the resulting biocompatible tissue for tendon or ligament in a defective tendon or ligament of the animal including the human.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP00/07461

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61L27/32, A61F2/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61L15/00-33/18, A61F2/00-4/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA(STN), MEDLINE(STN), WPI/L(QUESTEL)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	JP, 10-127752, A (Norin Suisansho Sanshi Konshu Nogyo Gijutsu Kenkyusho), 19 May, 1998 (19.05.98), Column 12, lines 36-47 (Family: none)	1-3 4-6,8
X Y	JP, 8-40711, A (Agency of Industrial Science and Technology), 13 February, 1996 (13.02.96), Claims; Column 1, lines 16-20; Column 3; example 2 (Family: none)	1-3 4-6,8
X Y	JP, 6-339521, A (TAKIRON CO., LTD.), 13 December, 1994 (13.12.94), Claim 9; Column 1, lines 38-46 (Family: none)	1-3 4-6,8
Y	JP, 11-171516, A (Agency of Industrial Science and Technology), 29 June, 1999 (29.06.99), Claims; Column 2, lines 20-26 & US, 6153266, A	4-6,8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January, 2001 (23.01.01)	06 February, 2001 (06.02.01)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP00/07461

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 9 pertains to methods for treatment of the human body by surgery or therapy.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)